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Environmental Contamination and Toxicology

Parasites of Fish as Biomarkers of Environmental Degradation: A Field Study

R. A. Khan

Department of Biology and Ocean Sciences Center, Memorial University of Newfoundland, St. John's, NL, Canada, A1B 3X9

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Several methods are currently used to assess stress-induced changes in animals exposed to pollutants in the aquatic environment, especially detoxication enzymes (Adams 1990). Other studies have suggested that since some parasites of fish are sensitive to environmental change, they can be useful as biomarkers of environmental health, biodiversity, changes in communities and also subtle intrinsic alterations in their hosts (Khan and Thulin 1991 and references therein; Khan and Payne 1997; Overstreet 1993; Yeomans et al. 1997; Khan 1998; MacKenzie 1999). Moreover, an analysis on the use of fish parasites as biomarkers revealed that ectoparasites were the most useful group since these responded positively to environmental change (Lafferty 1997). To test this hypothesis further, a number of studies were conducted recently to determine the mean abundance and prevalence of ectoparasites as biomarkers on two species of fish exposed naturally to xenobiotics including petroleum hydrocarbons (PHCs) originating from an oil refinery, effluent from a pulp and paper mill (PPE) which contain resin acids, polychlorinated biphenyls (PCBs) from a naval dockyard and untreated domestic sewage from two locations in Newfoundland. Reference samples for comparison were obtained downstream from the sites of the perturbations. The focal species in these field studies were the shorthorn sculpin (*Myoxocephalus scorpius*) and winter flounder (*Pleuronectes americanus*) as both reside inshore, are benthic, sedentary with limited foraging capacity, can be captured readily by SCUBA divers and have been shown to be sensitive indicator species (Khan et al. 1994 and references therein; Barker et al. 1994).

MATERIALS AND METHODS

Two species of fish were sampled at contaminated and reference sites for ectoparasites in coastal localities of Newfoundland. Sculpins and winter flounder were captured in spring by SCUBA divers using a net at depths of 5 to 10 m near an oil refinery at Come By Chance (47°46', 54°02' W), a sulphite-bleaching pulp and paper mill at Corner Brook (49°01' N, 58°02'W), a PCB-contaminated naval facility at Argentia (47°16'N, 53°59'W), an untreated domestic sewage outfall near a boat harbor at Bay Bulls (46°34'N, 52°58'W) and reference sites located five to 12 km farther away. Concentration of the contaminants are shown in Table 1, based on

reports by Kiceniuk (1993) and unpublished data by Environment Canada and Department of Public Works, Canada. No hydrocarbons or other contaminants were present at detectable levels in samples of the domestic sewage taken annually over the 5-year period. Winter flounder and sculpins, all mature female fish, varied in length from 21-27 cm and 26-32 cm respectively. In a five-year study on winter flounder inhabiting an area near an untreated sewage outfall in Trinity Bay (47°52'N, 53°55'W), 12 fish (25-30 cm, 4-5 years old, females only) each from this and two additional sites located about 5 and 10 km downstream, were sampled annually during summer. All fish were autopsied shortly after capture. Mucus from the skin/gills was removed with a 24mm^2 cover glass and the number of parasites enumerated by microscopic examination using a hemocytometer and expressed as the number/mm^2. Metacercariae of a digene, *Cryptocotyle lingua*, parasitised both the skin and gills of winter flounder. Since the abundance on the skin was too numerous to enumerate accurately, counts of the cysts infecting the gills was found to be a more useful method for estimation. Consequently, gill samples, from the second right branchial arch, were fixed in 10% buffered formalin, processed by conventional histological methods and stained with hematoxylin and eosin. The number of metacercariae encysted on the gills was enumerated following microscopic examination of 10 primary lamellae. A comparison was also made of hemosiderin deposits in the spleen between the fish groups. Samples of spleen were fixed and processed as mentioned previously and sections stained with Perl's Prussian blue. Hemosiderin was estimated by digitial image analysis and expressed as a percentage of the area (%/mm^2) scanned (Khan and Nag 1993). Since the data were not normally distributed, a non-parametric method of Kruskal-Wallis was used to test for significance. Means and standard errors or derivations were calculated for all fish groups and differences considered significant when $p<0.05$, using the SPSS software package. Mean abundance and prevalence of parasites follows the terminology proposed by Bush et al. (1997).

RESULTS AND DISCUSSION

Sculpins and winter flounder, sampled near locations where the pollutants occurred, exhibited a significantly greater mean abundance of parasites than reference sites (Table I). However, prevalence was also different but not consistently between contaminated sites and reference locations farther away. Mean parasitic abundance varied from 5 to 50 times greater in fish sampled at the contaminated locations which included an oil refinery, untreated effluent from the pulp and paper mill and PCBs near a naval facility than at reference sites. Higher mean abundance of both trichodinids on the skin and metacercariae of *C. lingua* on the gills were also observed in flounder taken near a untreated domestic sewage outlet than at a reference site. Species of *Trichodina* infecting the sculpin included *T. cottidarum* and *T. saintjohnsi* while *T. murmanica* and another undescribed species infected winter flounder. Secondary branchial lamellae of the parasitised sculpins and winter flounder, captured at the contaminated sites displayed evidence of hyperplasia culminating in fusion of adjacent filaments and an excessive mucus exudate. None

of these lesions was observed in fish taken from the reference sites. Distortion of the primary lamellae by multiple infections with metacercariae of *C. lingua* was also observed in sculpins and winter flounder captured at the polluted but not at the reference sites. Hemosiderosis occurred in fish sampled near the contaminated and reference sites. However, these deposits in the spleen, located in melano-macrophage aggregates, were significantly greater in fish examined at the contaminated than at reference locations (Table 1). These differences were greatest, at least five times more, in fish taken near the oil refinery, pulp and paper mill and the PCB-contaminated wharf than at the reference sites. No differences in hemosiderin concentration were observed in flounder sampled near the untreated sewage and reference site at Bay Bulls.

Since it appeared that sewage influenced the mean abundance of ciliates on the gills of the sculpin, an additional study was conducted on winter flounder in an inlet where only untreated domestic sewage was discharged and at two other locations downcurrent. Metacercariae, too numerous to count on the skin, occurred in fish taken near the outfall but were fewer substantially at the other two locations. The parasites were also encysted in the gills and counts revealed that the prevalence and mean abundance were significantly greater in the lamellae of samples taken near the outfall than at the two other sites (Fig. 1). Mean prevalence of *C. lingua* on the gills varied from 75-100% at site one, 42-80% at site two and were least, 13-25%, at site three. Similarly, the mean abundance near the sewer outlet (site 1) varied from 3.3-11.1, being somewhat lower at site two, 1.3-2.3, and least at site three, 0.2-1.6 cysts/lamella. Although mean abundance and prevalence at each site fluctuated between years, there appeared to be no clear evidence of an increasing or decreasing trend over the five year-period. Metacercariae of *C. lingua,* encapsulated as fibrotic cysts in the primary branchial lamellae of flounder sampled at all three sites, caused distortion more often in fish taken from site one, especially when multiple parasites were present. The latter induced the lamellae to assume an 's' shape caused by the lateral extension of the fibrotic capsule when the parasites occurred on opposite sides. No difference in the concentration of hemosiderin deposits in the spleen of the flounder was apparent between the three sites over the five-year period.

Results from the present study have revealed a significant difference in the mean abundance and prevalence of species of the ectoparasites, *Trichodina* spp. and /or metacercariae of *C. lingua*, in sculpin and winter flounder sampled at /near contaminated sites than at locations farther downstream. Similar observations were made on ectoparasites previously in sculpins and winter flounder exposed both naturally and after chronic exposure in laboratory experiments to these contaminants which included effluent from pulp and paper mills, an oil refinery and PCBs at a naval facility (Barker et al. 1994; Khan et al. 1994; Khan 1998, 1999; Moles and Norcross 1998). Moreover, the changes in parasitic levels were associated with pathological changes in gill morphology and an excessive deposition of hemosiderin both of which are indicative of chronic exposure to pollutants (Khan et al. 1994 and

Table 1. Comparison of prevalence (%) and mean abundance ($\bar{x}$ ± s.e.) of the ciliates, *Trichodina spp.* (T) and/or metacercariae (M) of a digenean, *C. lingua*, parasitising the gills (G) or skin (S) of the shorthorn sculpin (SHS) and winter flounder (WFl) sampled at various distances (km) from the point of discharge of contaminants with samples from reference sites. Concentration of hemosiderin (%/mm^2, $\bar{x}$ ± s.e.) in the spleen and the pollutants is included.

Type of pollutant	Fish species	Parasite (location)	Distance (~km)	n	% infected	Abundance $\bar{x}$ ± s.e.	Hemosiderin (%/mm^2)	Pollutant conc^n	Reference
COR†	SHS	T(G)	0.5	20	100	16.4 + 1.7	6.2 ± 0.3	>50 µg/g	Kiceniuk (1993)
〃	〃	〃	5.0	17	82	3.2 + 1.1*	1.1 ± 0.1*	–‡	
〃	WFl	T(S)	0.5	12	100	6.8 + 1.2	5.8 ± 0.3	>50 µg/g	〃
〃	〃	〃	5.0	12	100	0.9 + 0.3*	1.6 ± 0.2*	–	
〃	〃	M(S)	0.5	12	100	2.3 + 0.6$^+$	5.8 ± 0.3	>50 µg/g	〃
〃	〃	〃	5.0	12	22	<0.5*$^+$	1.6 ± 0.2*	–	
PPE	SHS	T(G)	0.5	12	83	26.0 + 1.8	9.4 ± 0.5	27 mg/L	Environ. Canada**
〃	〃	〃	12.0	14	44	0.4 + 0.2*	2.3 ± 0.2*	–‡	
PCBs	SHS	T(G)	0.5	10	100	26.9 + 1.9	8.6 ± 0.5	2.11-4.14 mg/g	Public Works, Canada
〃	〃	〃	10.0	11	100	2.7 + 0.6*	1.8 ± 0.2*	–	
〃	〃	M(G)	0.5	10	100	32.3 + 2.1$^+$	8.6 ± 0.5	2.11-4.14 mg/g	
〃	〃	〃	10.0	11	45	1.1 + 0.4*$^+$	1.8 ± 0.2*	–	
DS	WFl	T(S)	0.5	15	100	14.6 + 0.8	1.2 ± 0.3	–	
〃	〃	〃	5.0	15	33	0.6 + 0.2*	0.8 ± 0.1	–	
〃	〃	M(G)	0.5	15	100	6.1 + 2.8$^+$	1.2 ± 0.3	–	
〃	〃	〃	5.0	15	40	0.4 + 0.1*$^+$	0.8 ± 0.1	–	

†COR - crude oil refinery; PPE - pulp and paper mill; PCBs - polychlorinated biphenyls; DS domestic sewage
*Significantly different ($P < 0.05$) from comparison group
$^+$Same group of fish infected with *Trichodina spp.*
‡No detectable levels
**Unpublished data.

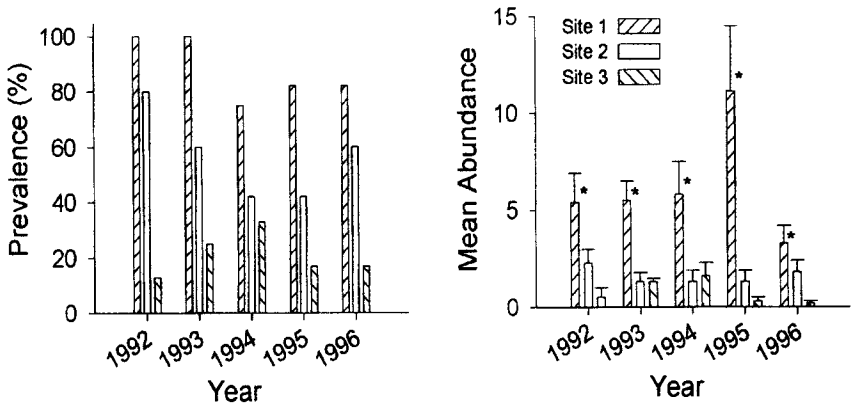

Figure 1. Prevalence and mean abundance ($\bar{x}$ ± s.d./10 primary lamellae) of metacercariae of *C. lingua* encysted on the gills of winter founder sampled near a domestic sewage outlet (no. 1) and two sites (nos. 2 and 3) located downstream over a 5-year period. Asterisk indicates significant differences between sites.

references therein). Several reports also indicate that ectoparasitic ciliates respond positively to environmental changes including industrial sewage, thermal effluent, heavy metals, organochlorines, etc. (Khan and Thulin 1991; Axelsson and Norrgren 1991; Khan et al. 1994; Broeg et al. 1999). In contrast, results were inconsistent when endoparasites were examined as potential bioindicators (Lafferty 1997; Marcogliese et al. 1998). It is evident, then, from the present and previous studies that ectoparasites are sensitive to environmental change and are useful as biomarkers (Khan and Thulin 1991; Yeomans et al. 1997; Marcogliese et al. 1998). A number of factors might have contributed to an increase of abundance in the present study. In sculpins and winter flounder, chronic exposure to the contaminants culminated in an excessive secretion of mucus, providing a habitat conducive for the ciliates to reproduce, and also probably suppressed the immune response simultaneously (Schreck 1996). Moles and Wade (2001) reported that exposure of sand lance (*Ammodytes hexapterus*) to crude oil-contaminated sediment for 90 days reduced phagocytic activity and was associated with a significantly greater abundance and prevalence of the ectoparasitic monogene, *Gyrodactylus* sp., than in the control group. The authors concluded that the PHC concentrations were adequate to suppress the immune system culminating in increased parasitism.

The present study has also noted that the prevalence and abundance of metacercariae of the digene, *C. lingua*, in the gills of winter flounder were significantly greater near the outfall of untreated domestic sewage than at two distant sites down-current. Moreover, the lowest prevalence, and occasionally abundance, occurred in fish at the most distant location. This gradient of response was probably associated with a greater abundance of its main intermediate host, the periwinkle *Littorina littorea*, infected with larval stages near the sewer outfall where fecal matter was disposed than at the other sites. Both *C. lingua* and the periwinkle are widely distributed in

coastal Newfoundland primarily wherever organic matter is disposed, such as near a sulphite- bleaching pulp and paper mill than in areas without similar infusions (Sekar and Threlfall 1970; Barker et al. 1994; Khan 1998). Siddall et al. (1993) reported that the number of larval digenes in another mollusc, *Buccinum undatum*, was greater near the sewer dumpsite and decreased with increasing distance from that location. In contrast to these observations, both the prevalence and abundance of *C. lingua* in flounder was significantly lower near a PCB-contaminated naval facility than at farther sites (Khan 1999). This difference was probably associated with toxicity of the xenobiotic to the periwinkle and also to the larval stages of the parasite.

In conclusion, the present study has shown that ectoparasitic trichodinid ciliates and metacercariae of *C. lingua* respond positively after chronic exposure to environmental contamination and can be useful as biomarkers. Moreover, the increase in prevalence and abundance of the parasites coincided with pathological changes suggestive of prolonged exposure. A study, conducted over a three-year period in the North Sea including the German Bight contaminated with the organochlorines, PCBs and DDT, revealed that the parasite species of flounder (*Platichthys flesus*) exhibited a gradient of responses in diversity between the polluted and reference sites (Broeg et al. 1999). Prevalence and mean abundance of *Trichodina* sp. infecting the gills, were significantly greater at the contaminated site than at three other less polluted reference sites. Moreover, parasitic levels correlated with elevated EROD activity, increase of neutral lipid deposition, decrease of lysosomal membrane stability, a decline of macrophage activity in males only and an accumulation of the metabolites of the organochlorines in both liver and muscle. These results provide further evidence in support of the view that ectoparasites can be useful as biomarkers of environmental degradation.

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